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CZECHOSLOVAK DEVICE FOR AUTOMATIC APPLICATION OF  
DETACTION REAGENTS ON CHROMATOGRAPHIC PAPER

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CZECHOSLOVAK DEVICE FOR AUTOMATIC APPLICATION OF  
DETECTION REAGENTS ON CHROMATOGRAPHIC PAPER

[Following is the translation of an article by Miroslav Steft in Chemicke Listy (Chemical Papers) Vol 54, No 11, November 1960, pages 1187-1189.]

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The quantitative distribution of materials classified on chromatographic paper employs several methods of applying the detection reagents to the paper.

Usually a solution of a detection reagent is passed through the chromatogram, for example, in determining amino acids in a solution of ninhydrine in acetone or in other solvents, or in determining sugar in methanol solution of triphenyltetrazoliumchloride, alkalized NaOH, and such. This method of application gives relatively small errors in the results of a quantitative determination of matter. Accuracy demands that the materials which are separated on the paper and products of the occurring reactions cannot be dissolved in the solution, which is often very difficult to achieve.

Another common method that uses a spray cannot achieve regularity in spreading the detection matter on paper, which leads to uneven tinting of the matter, especially where the reactions are not quantitative.

Other methods of applying detecting reagents to paper cannot be used successfully in determining the quantity of the matter.

In order to remove the deficiencies of the above-cited results, our laboratory has designed a device for the automatic application of detection reagents on chromatographic paper.

The basis of our applicator is a burette with a large number of capillary elimination outlets arranged in a row one after the other, 3 mm apart, through which the solution runs onto the paper. We used mineral glue

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(1 part ZnO & 1 part MnO<sub>2</sub> & 1.5 parts of water glass) to glue together the thick little capillary tubes, ground in the shape of little prisms 3x3x10 mm, of inside diameter 0.3 mm, and the resulting block we put into longitudinal slit in a rust-proof tube (dural, stainless steel) inner diameter 10 mm, and sealed it with the same glue. Into the tube we inserted a polyethylene tube of inner diameter 6 mm, fitting it tightly against its wall, which removed the possibility of metal corrosion by organic materials and the passage of the metals into the solution. The lower area of the capillaries was ground off to make it level. To one side of the tube was attached a cylindrical separating funnel 50 ml, to the other side a ground glass stop cock (plate 1).

This mechanism is attached to a stand on a board 90 cm long, whose width depends on the length of the block of capillaries. To regulate the speed of solution flow from the capillaries, a little tube was attached to the side of the applicator that was levelled off. On the upper end of the tube there is a capillary opening that is experimentally sealed up so as to permit the flow of 1 ml of solution per 10 cm<sup>2</sup> area of the paper with speed relative to the movement of the paper and viscosity of the solution. A sponge rubber board with a smooth surface capable of being raised and lowered by means of screws in the board of the mechanism is used to close the capillary openings when the burette is being filled (plate 2, part 4). After the capillary block is closed, the burette is filled in such a way that the solution poured into the separating funnel is let out by the opening of both of the cocks into the tube and the capillaries. After the cocks are closed, the rubber plate is lowered. So that the solution does not leak out of the burette, the capillary block has to be in a longitudinal position.

The chromatographic paper is passed along the lower part of the capillary block, so that the paper absorbs the solution that is flowing out. Even absorption requires that the paper forms a groove under the capillary block, and so siliconized glass rods are installed on each side of the block about three mm apart. Their upper edge is about three mm higher than the lower edge of the capillary block. In inserting the paper into the machine, the rods are lowered about two cm below the level of the edge of the block and raised only directly before the lowering of the conveyor of the paper by means of the weights attached to them (plate 2, parts 5 and 8). The paper passes through evenly by means of a phonograph electric motor with a gear that gives ten revolutions per minute. The axis of the gear has disks of various diameter around it. A metal arm

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with teeth is used to secure and manipulate the paper at an even height. The length is relative to the width of the paper clamped down by the spring (Plate 2, Parts 13 and 14). This process enables us to move the paper evenly at the necessary speed. We were able to move paper 10 cm long in 2-4 seconds. To synchronize the beginning of paper movement and solution flow from the capillaries, a switch is attached to the electric motor on the stop cock of the separating funnel; a turn of the cock starts the motor and lets the solution out at the same time. Another switch is attached to the side plate of the stand that lowers the motor, in case there is a need to move arm 13 without releasing any solution.

The paper with the absorbed solution falls behind the block of capillaries onto a net made of silon [Czech type of nylon] threads (Plate 2, Part 18).

For each chromatogram, the solution in the separating chamber is filled to the same level, which equalizes both the pressure on the column of solution and the speed of the flow. So that the difference of the height of the level before and after the solution is absorbed into the paper will be the least, it is well to use separating funnels of ample width.

The above-described device enables a quick and regular application of the various solutions of detecting reagents on the whole area of chromatographic paper within 5-10 seconds, which heretofore was possible only when a solution of the detecting reagent was passed through the chromatograph. It is also possible to use solvents, in which the detecting material or the products of reaction are slightly dissolved without having the material eluted from the paper. The advantage of the device is that per unit of space the required amount of the detection material, additional ingredients, buffers, etc. can be safely and evenly applied, which makes the requirements of quantitative methods in direct coloration of materials on chromatographic paper standard procedure.

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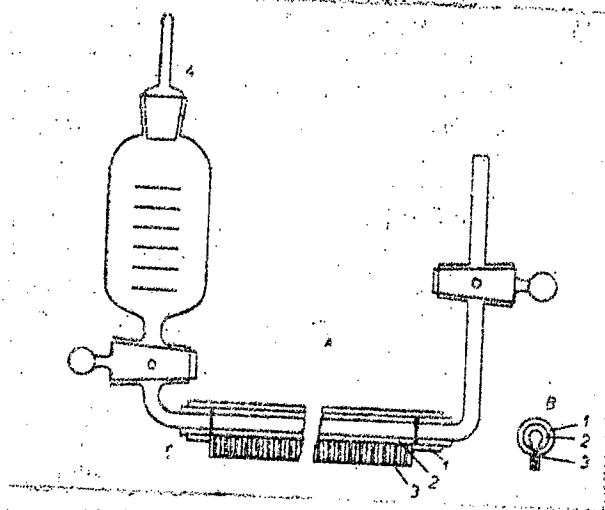


Illustration 1. Burette

- A. Front view
- B. Side view of the tube and the capillary block
1. Metal tube
  2. Polyethylene tube
  3. Block of capillaries
  4. Tube with a capillary opening
  5. Silicone rubber insulation

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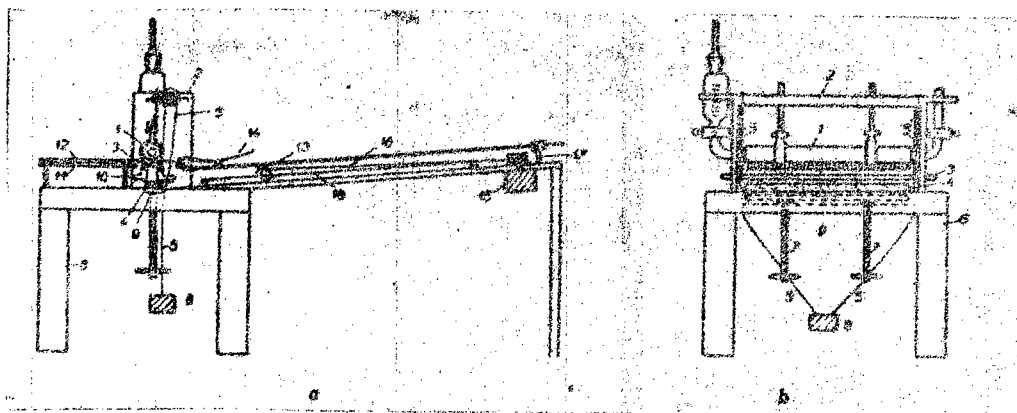


Illustration 2

Apparatus for Automatic Application of the Solution of  
Detection Reagents to Paper Chromatograms

a. Front view

b. Side view

1. Burette
2. Stand with burette clamps
3. Glass rods which guide the paper
4. Sponge rubber plate
5. Impenetrable net
6. Apparatus support
7. Screws for raising the sponge rubber locks
8. Weights for moving the rods (3)
9. Board which supports the sponge rubber (4)
10. View of the side wall of the stand of the instrument  
which directs the rods (3)
11. Chromatographic paper support
12. Chromatographic paper
13. Metal arm
14. Brace for the toothed clamp which catches the  
chromatographic paper
15. Electric motor with reducing gear for 10 revolutions  
per minute
16. Impenetrable net of the arm (13)
17. Support which directs the arm (13)
18. Silon thread net

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END